β-COP Localizes Mainly to the *cis*-Golgi Side in Exocrine Pancreas

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Abstract. We examined the distribution of the non-clathrin-coated vesicle-associated coat protein β-COP in rat exocrine pancreatic cells by immunogold cytochemistry. Labeling for β-COP was found in the Golgi region (48%) where it was associated with vesicles and buds of ∼50 nm, showing a characteristic ∼10-nm-thick coat. The other half of the label was present in the cytoplasm, not associated with visible coats or membranes, with a minor fraction present on small clusters of tubules and vesicles. Clathrin-coated vesicles were typically located at the *trans*-side of the Golgi complex, and showed a thicker coat of ∼18 nm. Of the total β-COP labeling over the Golgi region, 68% occurred on the *cis*-side, 6% on the cisternae, 17% on the rims of the cisternae, and only 9% on the *trans*-side. For clathrin these figures were 16, 2, 4, and 78%, respectively. At the *cis*-Golgi side β-COP was present in transitional areas (TA), on so-called peripheral elements (PE), consisting of tubules and vesicles located between the cup-shaped transitional elements (TE) of the RER and the *cis*-most Golgi cisternae. Label for Sec23p was also present in TA but was located closer to the TE, while β-COP labeled

PE were located near the *cis*-Golgi cisternae. Upon energy depletion, Golgi associated β-COP was almost exclusively (86%) in spherical aggregates of 200–500 nm in diameter, whereas the *cis*-side (6%), the cisternae (1%), the rims (4%) and *trans*-side (3%) of the Golgi complex, were barely labeled; 50% of the total label remained in the cytoplasm. The aggregates were predominantly located at the *cis*-side of the Golgi stack, next to, but distinct from the Sec23p positive TA, that were devoid of β-COP and had only a few recognizable vesicles left. Incubation with aluminum fluoride resulted in fragmentation of the Golgi complex into large clusters of β-COP positive vesicles, while 50% of the label remained in the cytoplasm, as in control cells. After 10 min of Brefeldin A treatment 91% of β-COP was cytoplasmic and only 7% associated with membranes of the Golgi complex. The total label for β-COP over exocrine cells remained unchanged during the incubation with either of the drugs, indicating that the drugs induce reallocation of β-COP. Our data suggest that β-COP plays a role in membrane transport at the *cis*-side of the Golgi complex.

T HE regulated secretory pathway in exocrine pancreatic cells has been well described (Palade, 1975). Newly synthesized proteins exit the RER near the Golgi stacks at smooth-surfaced cisternae, so-called transitional elements (TE).[1] Between the TE and the *cis*-side of the Golgi stacks many vesicles and tubules occur, the peripheral elements (PE). Clusters of PE are found in areas, the transitional areas (TA), which are largely surrounded by TE. The occurrence of protuberances at the concave TE membrane suggest the formation of PE, which provide for the vesicular transport of newly synthesized secretory proteins between RER and Golgi stack (Jamieson and Palade, 1967; Slot et al., 1976, 1983). In other cell types structures comparable

with the PE in pancreatic cells, have been named pre-Golgi vacuoles (Saraste and Kuismanen, 1984; Saraste et al., 1987), salvage compartment (Warren, 1987), budding compartment (Tooze et al., 1988), intermediate compartment (Schweizer et al., 1988, 1990, 1991), and *cis*-Golgi network (Hsu et al., 1991; Pelham, 1991; Rothman and Orci, 1992). Lotti et al. (1992), described an intermediate compartment between RER and Golgi complex in vesicular stomatitis virus (VSV)-infected Vero cells consisting of clusters of tubules and vesicles, that transport the viral glycoprotein G.

Pancreatic secretory proteins leave the Golgi stack at the *trans*-Golgi reticulum (TGR) where they are packed into secretory granules for regulated secretion. Though less prominent in pancreatic cells several other Golgi exits occur. The constitutive pathway to both the apical and basolateral plasma membrane is probably taken by the main pancreatic glycoprotein GP2, which explains its continuous and relatively fast appearance at the cell surface (Havinga et al.,

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1. *Abbreviations used in this paper:* AlF$_4^-$, aluminum fluoride; BfA, Brefeldin A; COP, coat protein; PDI, protein disulphide isomerase; PE, peripheral elements; TA, transitional areas; TE, transitional elements; TGR, *trans*-Golgi reticulum.
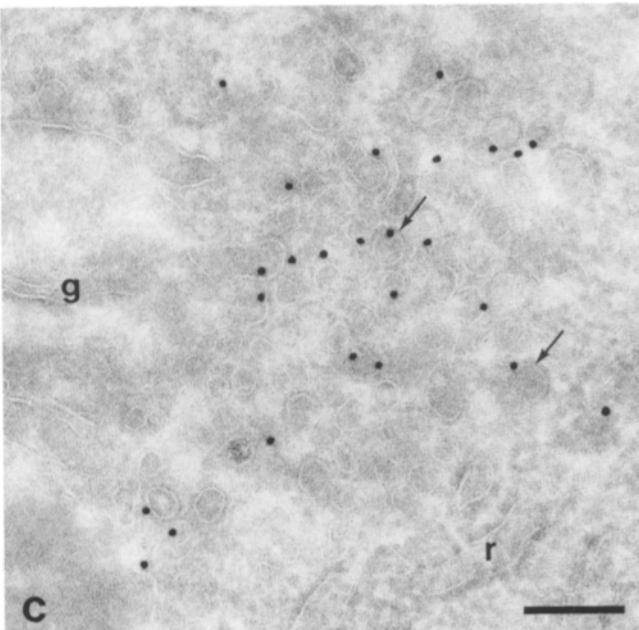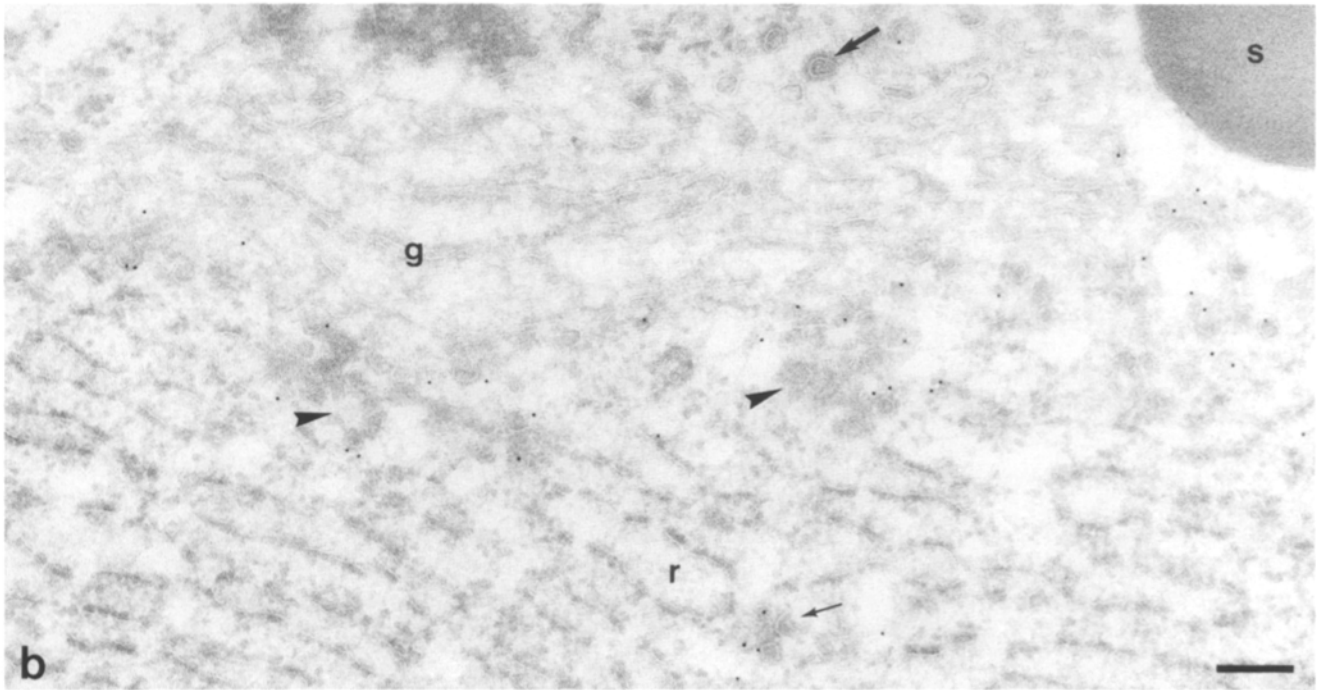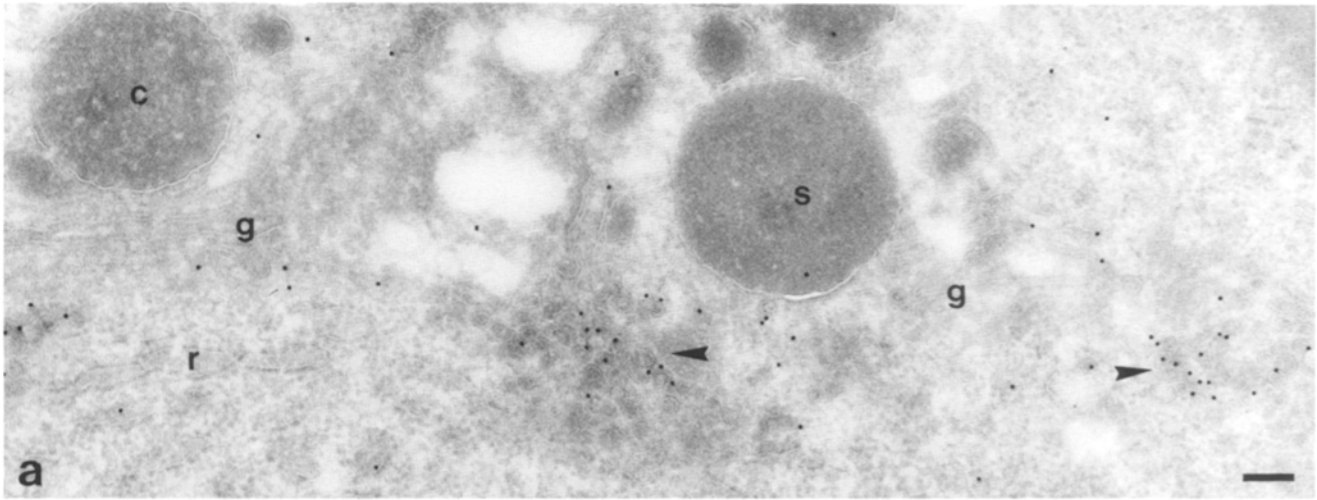
Figure 1. Distribution of β-COP in the Golgi region of normal rat exocrine pancreatic cells. (*a*) Gold labeling (15 nm) is most abundant on clusters of PE (*arrowheads*) located at the *cis*-side of the Golgi stack (*g*), some gold particles are present at the rims of the cisternae and at the *trans*-side of the stack. In *b*, a small cluster of vesicles in the region of the RER is labeled (*small arrow*), and a clathrin-coated bud is seen on the *trans*-side (*large arrow*). The arrowheads indicate β-COP labeled PE (10 nm gold). (*c*) Higher magnification of vesicles and tubules constituting PE. The PE show a 10-nm-thick cytoplasmic coat (*arrows*), positive for β-COP (15 nm gold). *c*, condensing vacuole; *s*, secretory granule; and *r*, RER. Bars, 200 nm.
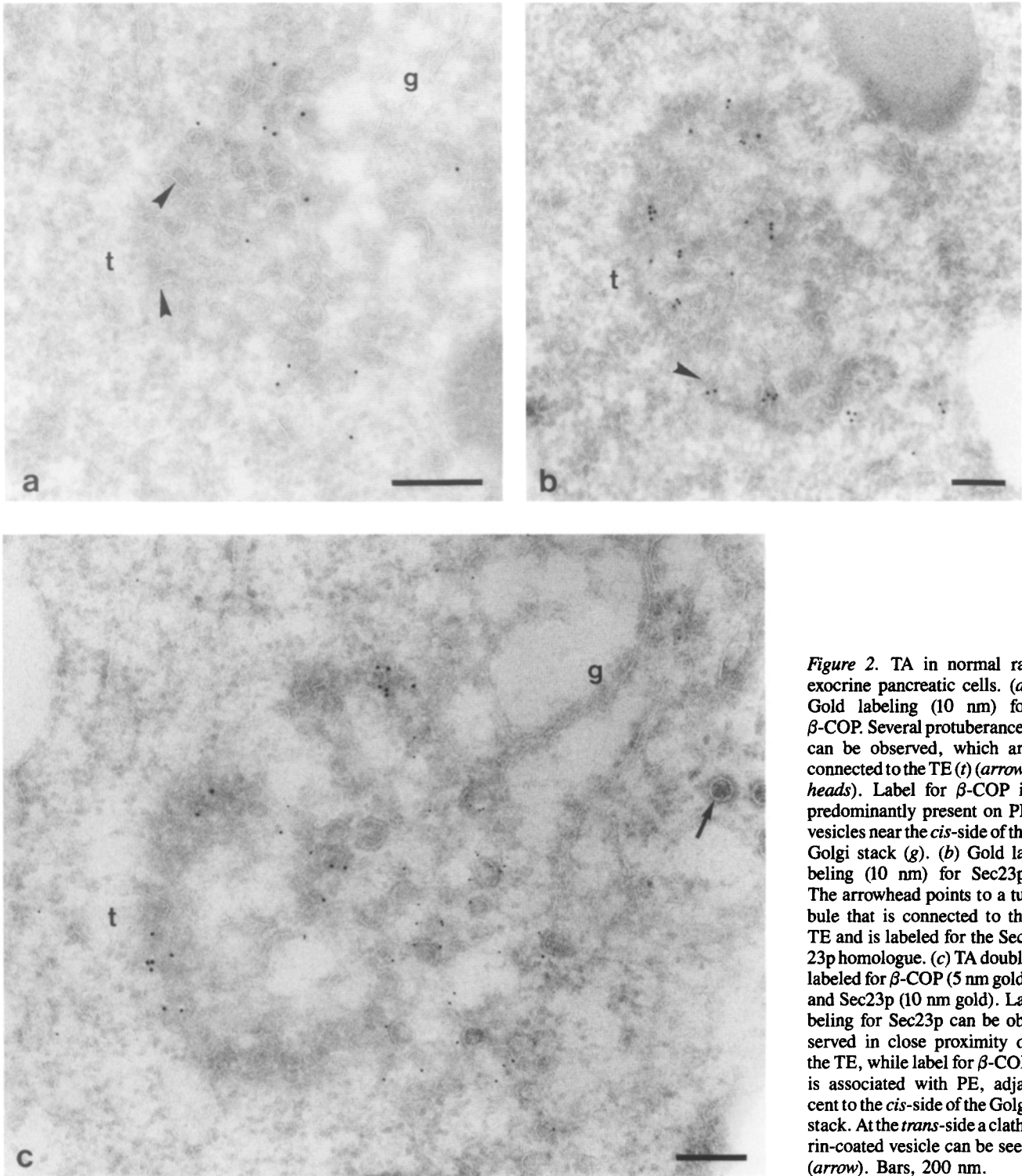
Figure 2. TA in normal rat exocrine pancreatic cells. (*a*) Gold labeling (10 nm) for β-COP. Several protuberances can be observed, which are connected to the TE (*t*) (*arrowheads*). Label for β-COP is predominantly present on PE vesicles near the *cis*-side of the Golgi stack (*g*). (*b*) Gold labeling (10 nm) for Sec23p. The arrowhead points to a tubule that is connected to the TE and is labeled for the Sec-23p homologue. (*c*) TA double labeled for β-COP (5 nm gold) and Sec23p (10 nm gold). Labeling for Sec23p can be observed in close proximity of the TE, while label for β-COP is associated with PE, adjacent to the *cis*-side of the Golgi stack. At the *trans*-side a clathrin-coated vesicle can be seen (*arrow*). Bars, 200 nm.

1983). Furthermore, newly synthesized soluble lysosomal enzymes are transport from the TGR (and the plasma membrane) to endocytic compartments, via clathrin-coated vesicles in which they are sorted due to specific recognition signals (Kornfeld and Mellman, 1989; Pearse and Robinson, 1990). These clathrin coats consist of two protein complexes, clathrin and adaptors, both also present in the cytoplasm as separate soluble pools (Pearse and Robinson, 1990). HA1, the Golgi adaptor, is a heterotetramer of poly-

peptides including γ-adaptin. The plasma membrane adaptor HA2 is a similar complex with α-adaptin. Adaptors are thought to bind to clathrin at one side and to the cytoplasmic domains of selected membrane proteins at the other (Pearse, 1988). Thus, mannose-6-phosphate receptors bind to both HA1 and HA2, while LDL receptors only have affinity for the HA2 adaptor (Kornfeld and Mellman, 1989).

Another class of coat proteins (COPs) constitute the cytoplasmic coat of a distinct class of carrier vesicles. COPs are
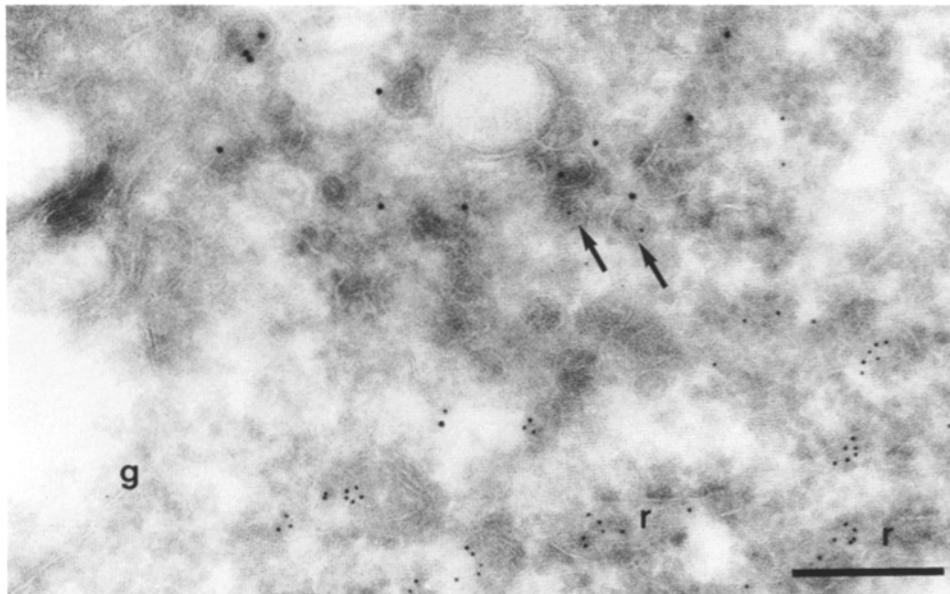
Figure 3. A Golgi area showing PE positive for β-COP (10 nm gold) and RER cisternae labeled for the resident RER protein PDI (5 nm gold). Occasionally, co-localization of both antigens is observed in vesicles (*arrows*). *g*, Golgi complex; *r*, RER. Bar, 500 nm.

less well characterized than the proteins of the clathrin-coated vesicles. Recently four COPs (α, β, γ, and δ) have been identified, which are found on nonclathrin-coated vesicles (Serafini et al., 1991; Kreis, 1992) and in a cytosolic complex (Waters et al., 1991). β-COP shares homology with β- and β'-adaptin (Duden et al., 1991). Although COPs are thought to be involved in transport of newly synthesized proteins to and through the Golgi stack (Orci et al., 1986; Mal-horta et al., 1989), it is unknown which vesicular transport steps in the Golgi stack are mediated by COP-coated vesicles.

Here we describe the steady-state distribution of β-COP relative to clathrin, at the electron microscopical level in rat exocrine pancreatic cells. We find the majority of β-COP present on the *cis*-side of the Golgi complex, while clathrin is primarily present on the *trans*-side. We also describe the effect of protein transport arresting conditions on the distribution of β-COP.

Materials and Methods

Tissue Processing

Male Wistar rats were used in this study and perfusion fixation through the

left ventricle of the heart was performed to obtain normal pancreatic material. For incubation experiments animals were decapitated, the pancreas was quickly taken out, mounted on a solid wax plate, and MEM tissue culture medium was injected into the interstitium, so that separate lobules could easily be dissected (Scheele and Palade, 1983). The medium was supplemented either without drug (control), with 1 μg/ml Brefeldin A (BfA), with 50 μM AlCl$_3$ and 30 mM NaF (AlF$_4^-$), or with 0.05% sodium azide and 50 mM 2-deoxyglucose (energy depletion, $-$E). The lobules were incubated at 37°C under 5% CO$_2$, for various periods of time from 0 to 40 min. At the desired time lobules were collected and fixed in a mixture of 2% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer. The lobules were kept in fixative overnight and were then transferred to 2% paraformaldehyde.

Sectioning and Antibodies

Single lobules were prepared for cryosectioning as described (Slot et al., 1988). Briefly, they were infused with 2.3 M sucrose and frozen in liquid nitrogen. Ultrathin cryosections were collected on copper grids and incubated under standard conditions. The following polyclonal antibodies were used: α-EAGE against β-COP (Duden et al., 1991), α-Clat R461 against clathrin light chain (kindly provided by E. Ungewickell, Max-Planck Institüt für Biochemie, Munich, Germany) and α-Sec23p (kindly provided by R. Schekman, University of California, Berkeley, CA) against yeast Sec23 protein (Orci et al., 1991). The mAb ID3 against the RER resident protein PDI (protein disulphide isomerase) was used to mark the RER (kindly provided by S. Fuller, EMBL, Heidelberg, Germany). The antibody binding sites were visualized with protein A/gold particles. Single and double labeling experiments were performed as described before (Slot et al., 1991). After gold labeling grids were treated with 1% glutaraldehyde. The sections were then contrasted with 3% uranyl acetate-oxalate, pH 7, and embedded in 1.8% methyl cellulose containing 0.3% uranyl acetate.

Table I. β-COP Distribution in Rat Exocrine Pancreatic Cells

	Relative distribution			
	Cytoplasm*	Golgi complex	Nucleus	Gold/μm²‡
Control	50.2 ± 1.0	48.2 ± 1.0	1.6 ± 0.2	0.71 ± 0.01
$-$E	50.5 ± 1.4	48.1 ± 0.5	1.4 ± 0.3	0.70 ± 0.02
AlF$_4^-$	52.4 ± 1.1	46.1 ± 1.0	1.5 ± 0.3	0.73 ± 0.02
BfA	90.6 ± 1.4	7.0 ± 1.2	2.4 ± 0.4	0.73 ± 0.02

Cells were either untreated (control), or energy depleted ($-$E), or treated with AlF$_4^-$, or BfA. Values are depicted as the mean (±SEM) of nine grids (see Materials and Methods).
* Except mitochondria and Golgi complex.
‡ Total of gold particles expressed per μm² of exocrine cell in the area counted.

Table II. Percentages of Immuno-gold Label for β-COP in the Golgi Region of Untreated (Control) and Energy-Depleted ($-$E) Rat Pancreatic Cells

	Buds and vesicles‡				
	Aggregates*	cis	lateral	trans	Cisternae
Control	–	68.2 ± 5.2	16.7 ± 4.1	8.7 ± 1.7	6.4 ± 1.4
$-$E	85.7 ± 3.9	6.1 ± 1.2	3.8 ± 2.1	3.2 ± 1.3	1.3 ± 0.2

Values are depicted as the mean (±SEM) of 12 Golgi regions (see Material and Methods).
* Mostly located on the *cis*-side of the Golgi complex.
‡ Buds and vesicles were classified according to their location, either on the *cis*-side (*cis*), lateral rims (*lateral*) or trans-side (*trans*) of the stacks of Golgi cisternae.
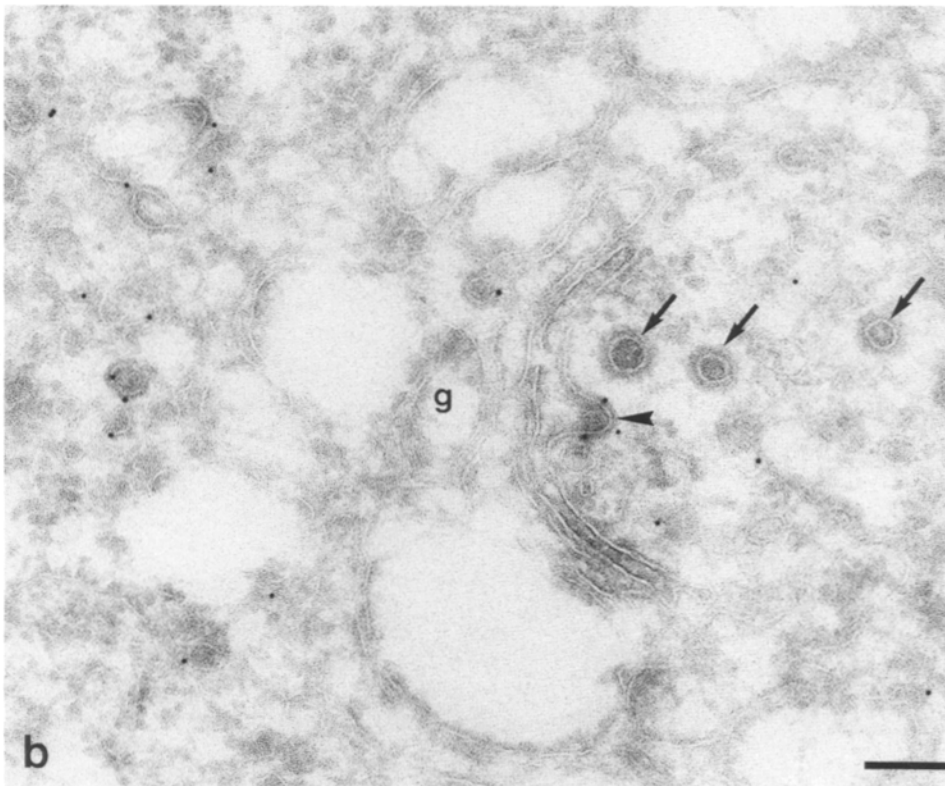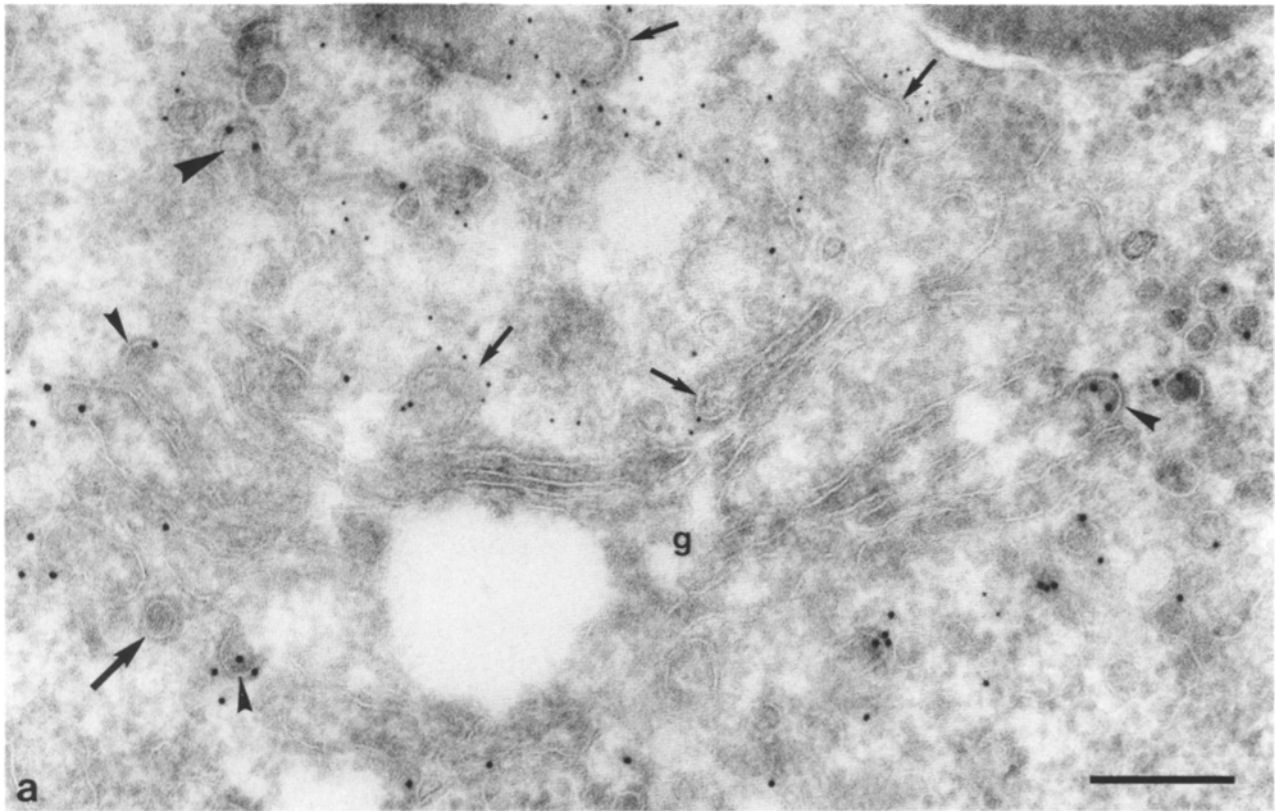
Figure 4. Localization of clathrin and β-COP in the Golgi region. (*a*) Double immunogold labeling for clathrin light chain (5 nm gold) and β-COP (10 nm gold). Clathrin is present on buds and vesicles (*small arrows*) on the *trans*-side of the Golgi stack (*g*), while β-COP mainly localizes to buds (*small arrowheads*) and vesicles at the *cis*-side and rims of the Golgi stack. Occasionally a β-COP positive bud is located at the *trans*-side (*large arrowhead*) and a (non-labeled) clathrin-coated vesicle can be observed on the *cis*-side (*large arrow*). (*b*) Single labeling for β-COP (10 nm gold), showing label on coated buds and vesicles at the *cis*-side, of the Golgi stack, and on an occasional bud at the *trans*-side (*arrowhead*), in close proximity to clathrin-coated vesicles (*arrows*). Note the difference in thickness between the coats of the two types of vesicles. Bars, 200 nm.

Quantitation

The relative distribution of label was calculated by randomly counting of immunogold particles over stretched ultrathin cryosections. Three locations were examined: the Golgi region, the rest of the cytoplasm (mitochondria excluded), and the nucleus. The level of labeling over the nucleus was taken as an indication for the background labeling. Simultaneously we determined the total number of gold particles present per surface area of tissue. We evaluated three different grids, obtained from three separate experiments. For quantitation of the distribution of label for β-COP and clathrin in the Golgi region, 12 micrographs showing cross-sectioned Golgi stacks from at least three different grids, were made at a final magnification of 42,800.
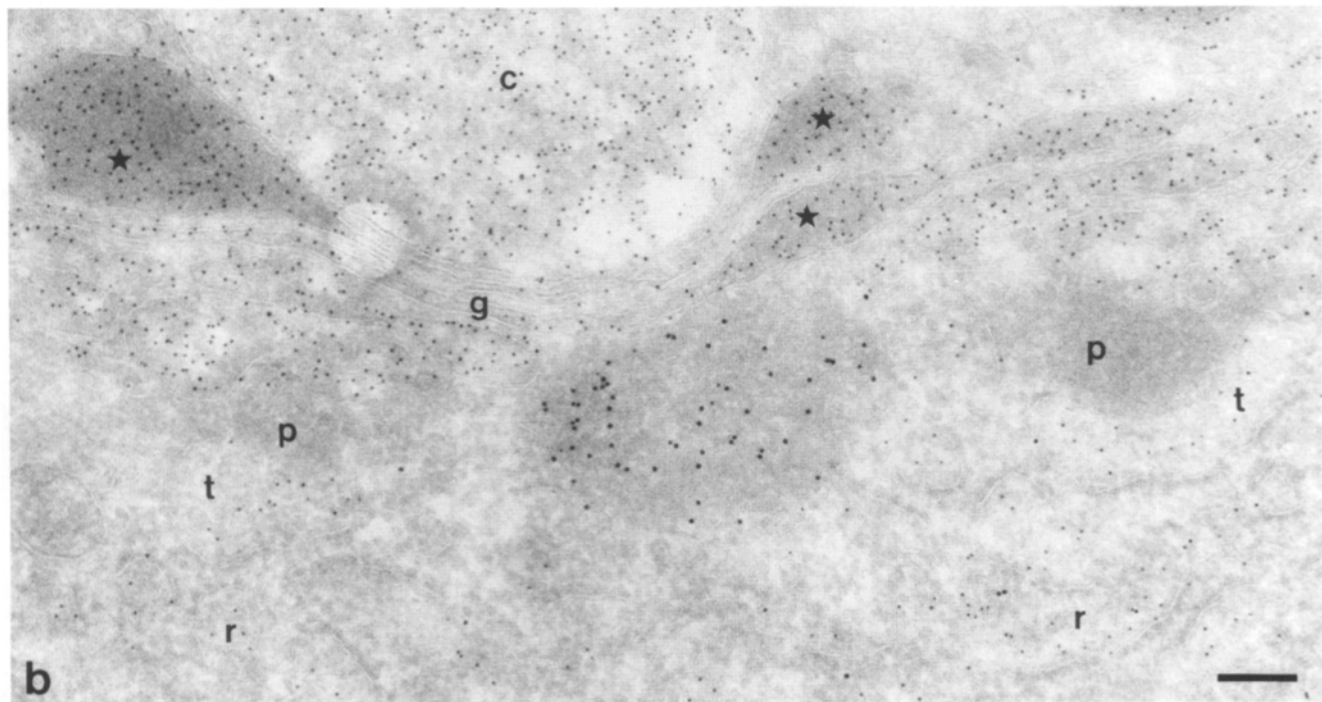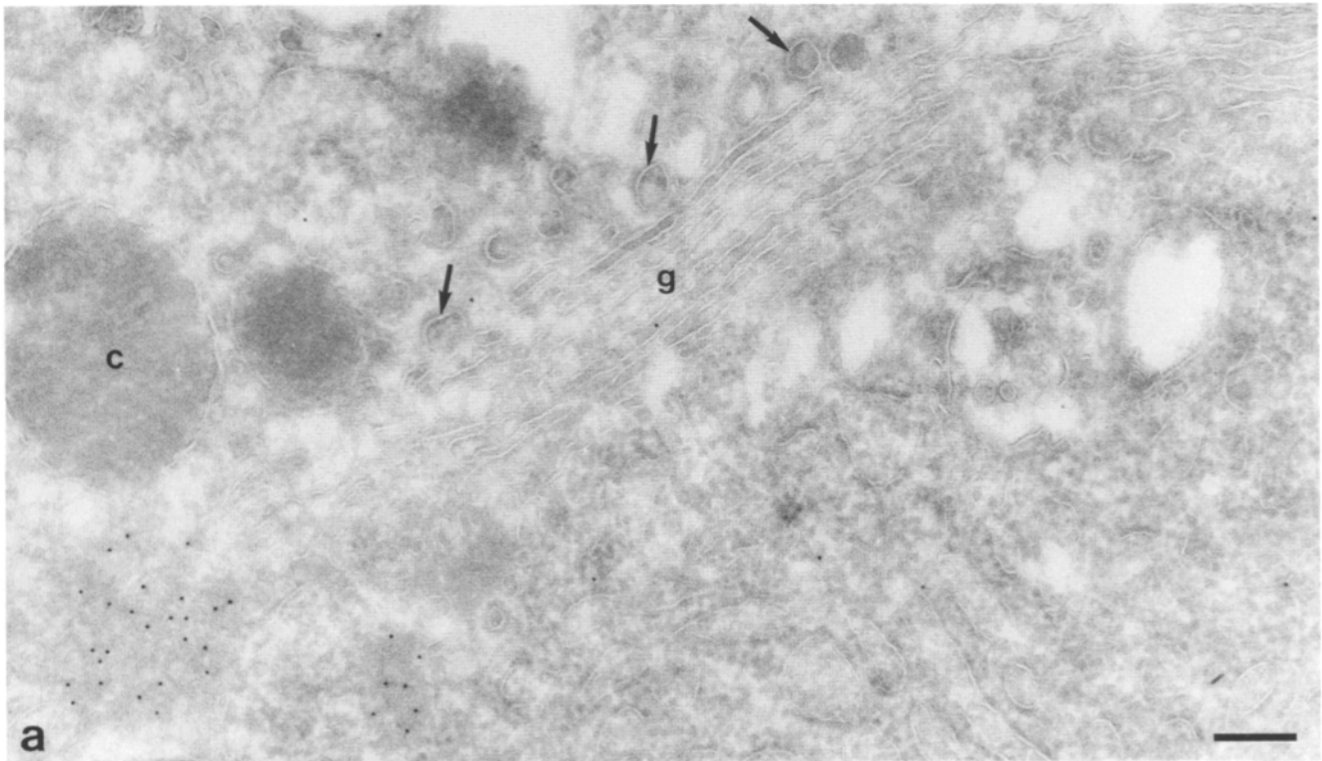
Figure 5. Effects of a 10-min energy depletion on the distribution of β-COP. (*a*) β-COP labeling is predominant in aggregates located on the *cis*-side of the Golgi stack (*g*), while clathrin-coated buds and vesicles (*arrows*) are present on the *trans*-side. Note the orderly stacked Golgi cisternae under these conditions. (*b*) Double immunogold labeling with anti–β-COP (10 nm gold) and a mixture of antisera against amylase and chymotrypsinogen (5 nm gold), showing that these secretory proteins are present inside RER (*r*) and TE (*t*) but not in the β-COP labeled aggregate and in the TA (*p*) in which only a few remnants of PE are left. Within the Golgi stack label is mainly present in marked areas (*asterisks*). *c*, condensing vacuole. Bars, 200 nm.

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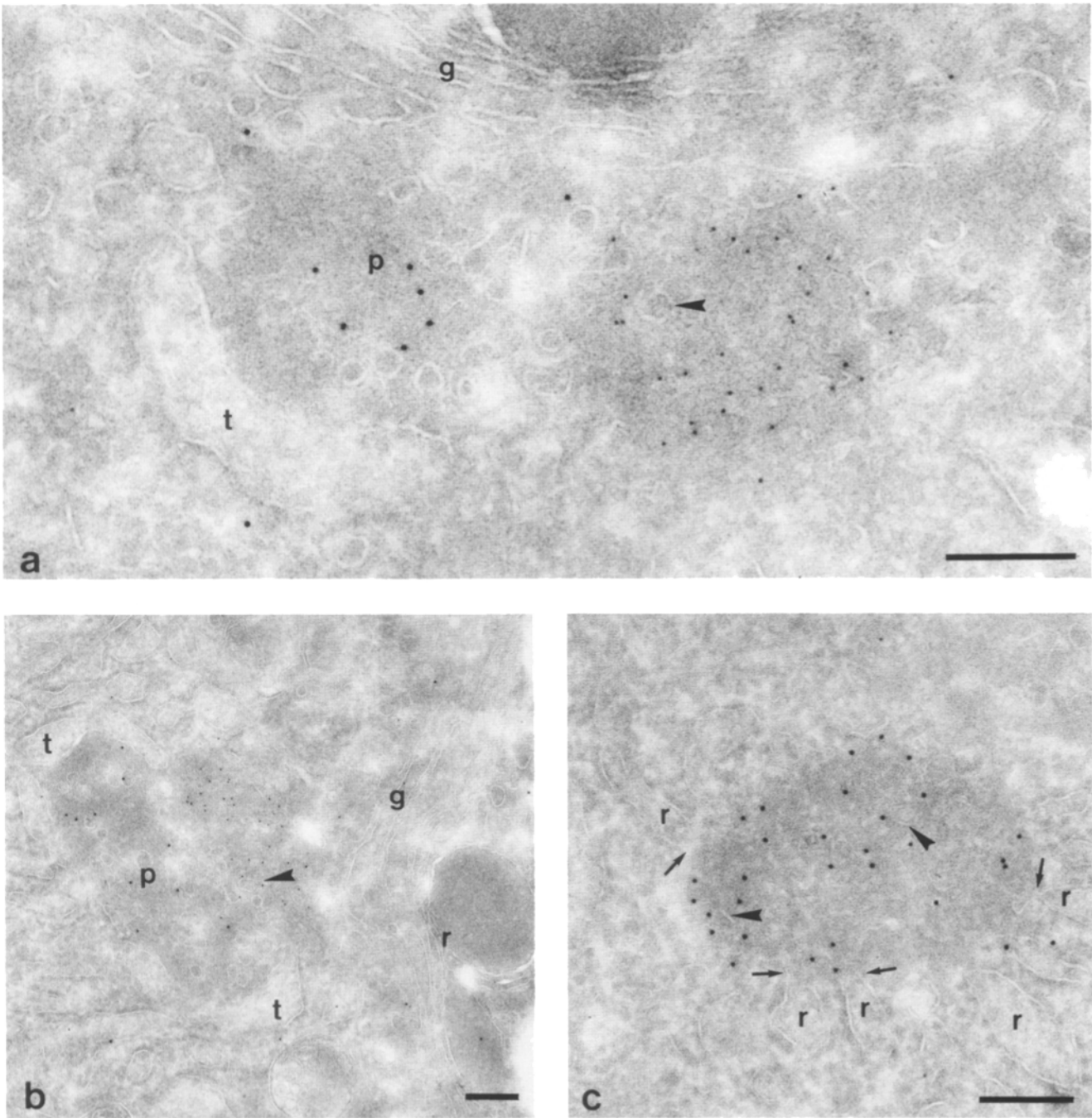
Figure 6. Localization of β-COP and Sec23p after a 10-min energy depletion. (*a* and *b*) Double-labeling for β-COP (5 nm gold) and Sec23p (10 nm gold). In *a*, β-COP is present in aggregates of electron dense material and segregated from a TA (*p*) which is labeled for Sec23p. The TE (*t*) are swollen, while the PE are less elaborate. In *b* a β-COP–positive aggregate is closely connected to a TA in which remnants of PE are still obvious. In *c*, RER cisternae (*r*) can be seen that are connected to a β-COP aggregate (*arrows*). The aggregates sometimes contain membranes (*arrowheads*). *g*, Golgi complex. Bars, 200 nm.

All quantitations were on tissue fixed after a 10-min incubation in the absence or presence of drugs (BfA, AlF$_4^-$, or −E).

Results

The Distribution of β-COP in Normal Rat Exocrine Pancreatic Cells

We investigated the steady-state distribution of the nonclathrin-coated vesicle-associated coat protein β-COP, in rat exo-

crine pancreatic cells by semi-quantitative immunogold cytochemistry on cryosections. β-COP labeling was predominantly found in the TA at the *cis*-Golgi sides and was associated with PE, which existed of tubules and vesicles of ∼50 nm in diameter (Fig. 1, *a* and *b*). Many of the PE were covered by a characteristic ∼10-nm-thin coat (Fig. 1 *c*). The PE consisted of both a delicate network of thin tubules connected to the TE, and vesicles with thin coats (Fig. 2). β-COP was associated with clusters of PE adjacent to the *cis-*
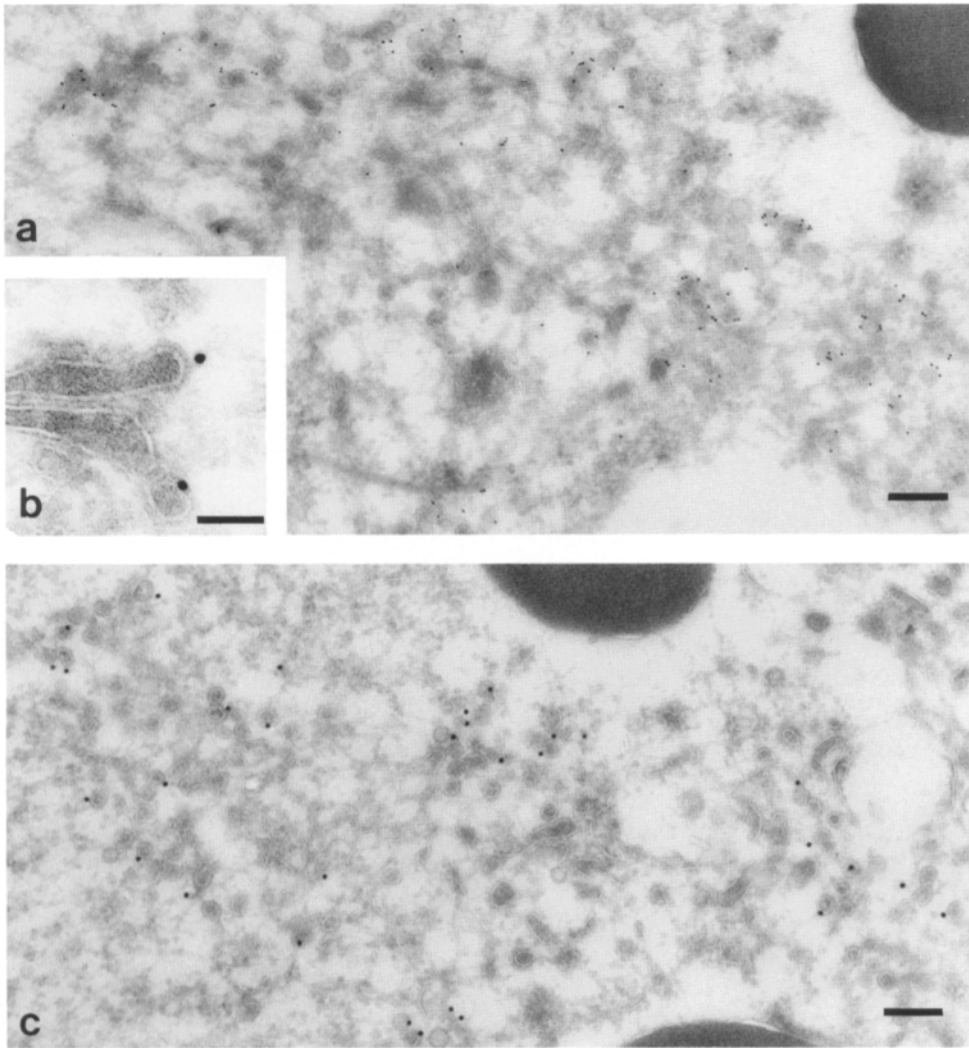
Figure 7. Effects of AlF₄⁻ on pancreatic cells. (*a*) After 10 min of treatment with AlF₄⁻, numerous β-COP–coated buds and vesicles (5 nm gold) appear and (*b*) Golgi cisternae show β-COP–coated buds (10 nm gold) on their rims. (*c*) Longer incubation (20 min) with AlF₄⁻ results in a more complete fragmentation of the Golgi complex and in an accumulation of β-COP–coated vesicles (10 nm gold). Bars: (*a* and *c*) 200 μm; (*b*) 100 μm.

Golgi cisternae (Fig. 2 *a*), while the Sec23 protein (reported to occur at the PE; Orci et al., 1991) was mainly present at the concave side of the TE (Fig. 2 *b*). In double-label experiments β-COP and the Sec23p homologue clearly localized to different subdomains of the TA (Fig. 2 *c*). The resident RER protein PDI occasionally colocalized with β-COP to the PE (Fig. 3). Labeling for PDI in TE is comparable in density with that in the RER. Apart from the Golgi region, β-COP labeling was also found associated with small clusters of vesicles and tubules throughout the cytoplasm (Fig. 1 *b*). These clusters have been described before (Jamieson and Palade, 1967; Geuze and Kramer, 1974) and are similar in structure to the PE, with which they have the cytochemical osmication reactivity in common (Slot and Geuze, 1979). They also show the characteristic 10-nm-thick coat of the COP-coated vesicles. Disperse β-COP labeling, not connected to any coat or membrane structure was observed in the cytoplasm. Some β-COP was also associated with filaments in the apical cytoplasm and with cellular junctions (not shown). Quantitation of β-COP labeling revealed that 48% was present on membranes associated with the Golgi complex and that 50% was randomly distributed over the cytoplasm (Table I). Of the Golgi label, 68% was present on vesicles and tubules at the *cis*-(Sec23 positive) side of the Golgi (Table II).

Clathrin was preferentially associated with vesicles and buds at the *trans*-side of the Golgi complex (Fig. 4 *a*), including the forming secretory granules. A minor but consistent fraction of clathrin-coated vesicles was present at the *cis*-side, usually in the direct vicinity of (pre-)lysosomal structures (data not shown). Clathrin labeling was associated with membranes covered by a coat of ∼18 nm thick, clearly distinguishable from the 10-nm-thick COP coat (Figs. 2 *c* and 4 *b*). Quantitation of the label for clathrin in the Golgi region showed that 78% was present on the *trans*-side of the Golgi stack, 15% on the *cis*-side, 4% on the rims, and 2% on the cisternae.

Effects of Energy Depletion

Upon energy depletion (10 min or longer) the distribution of β-COP dramatically changed. Distinct β-COP-positive aggregates of electron-dense material appeared at the *cis*-side of the Golgi complex (Fig. 5 *a*), located next to, but distinct from TA (Fig. 5 *b*) which were still marked by the Sec23p homologue (Fig. 6, *a* and *b*). The PE were now much less developed and the cup-shaped TE, which were clearly swollen under this condition (Fig. 6 *a*), now enclosed a TA mainly consisting of floccular ground substance (Figs. 5 *b* and 6 *a*), instead of the tubules and vesicles found in control
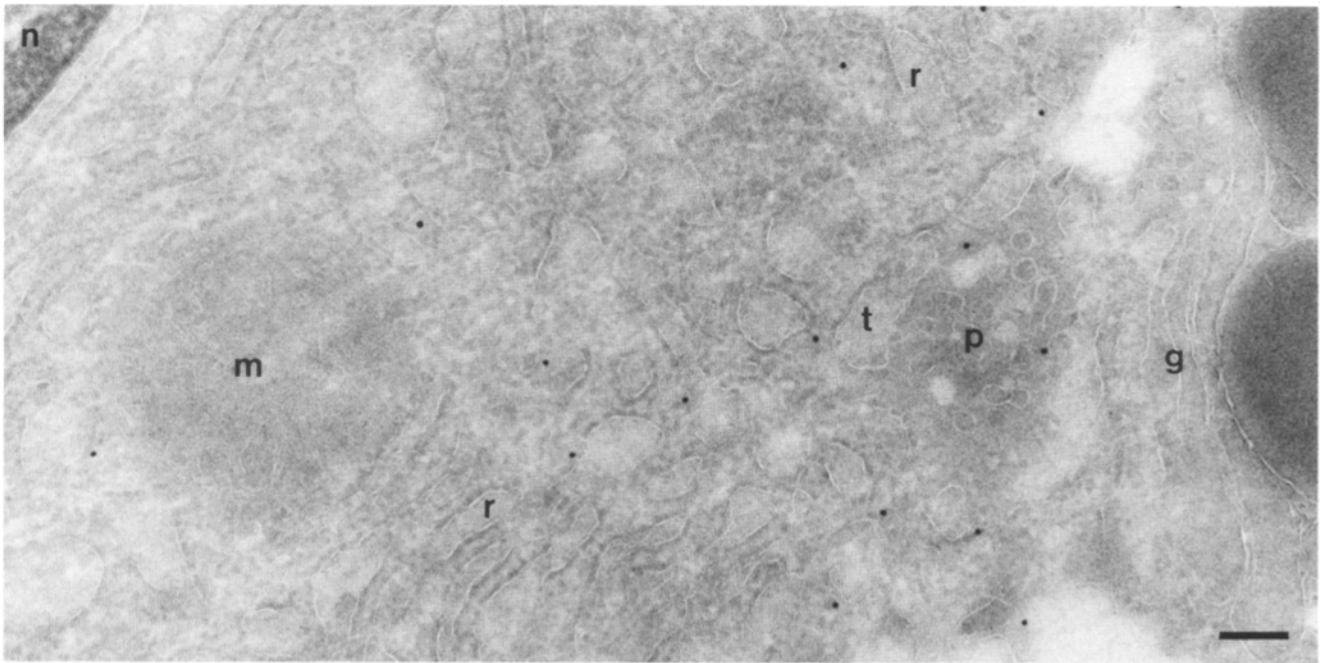
Figure 8. Incubation of pancreatic cells for 10 min with BfA. Low magnification showing the reallocation of β-COP (15 nm gold) from the peripheral elements (*p*) near the Golgi complex (*g*) to the cytoplasm. *t*, transitional element; *r*, RER; *m*, mitochondrion; and *n*, nucleus. Bar, 200 nm.

cells (see Fig. 2). Occasionally intermediate situations were encountered at 10 min, in which TE were still appreciable and the separation of aggregates from the TA was not complete (Fig. 6 *b*). Secretory proteins were detected in TE, but not in the TA or in the β-COP positive aggregates (Fig. 5 *b*).

Of the Golgi complex associated β-COP labeling 86% was present in the aggregates outside the TA (Table II). The aggregates had a diameter of 200–500 nm which is in the same size range as the TA under this condition. Their similar appearance (Fig. 6 *a*) may explain why both types of bodies were not distinguished in previous studies (Merisko et al., 1986*a*; Orci et al., 1991), but we noticed some differences. The ground substance of TA and aggregates differed slightly, the TA having a more fine texture (Fig. 6 *b*). The aggregates often contained some irregular membranous material (Fig. 6), and showed continuities between surrounding RER cisternae and membranes inside the aggregates (Fig. 6 *c*). The Golgi complex appeared more orderly stacked than in control cells (Fig. 5 *a*) and the cisternae were less filled with secretory proteins (i.e., amylase and chymotrypsinogen). This is probably due to the fact that most of the secretory proteins concentrated in distinct foci (Fig. 5 *b*). Quantitation revealed that the total β-COP labeling density of the cells had not changed by this treatment (Table I), and that still half of total β-COP was present in the Golgi area. Double labeling for clathrin and β-COP showed that clathrin remained associated with the *trans*-side of the Golgi (data not shown). There was no indication of clathrin aggregation after energy depletion, instead clathrin-coated membranes were even more obvious in the TGR than in control cells (Fig. 5 *a*).

Effects of Aluminum Fluoride AlF₄⁻

AlF₄⁻ is known to activate trimeric G-proteins and to enhance β-COP association to Golgi-membranes (Donaldson

et al., 1991). Incubation of pancreas tissue with AlF₄⁻ resulted in the accumulation of β-COP–coated buds and vesicles (Fig. 7 *a*). Already after 10 min of incubation with AlF₄⁻, more Golgi cisternae could be observed that contained β-COP-coated buds on their rims (Fig. 7 *b*). At longer incubation times this resulted in a loss of stacked Golgi cisternae (Fig. 7 *c*). Quantitation revealed that after 10 min control levels of the β-COP were present on coated buds and coated vesicles in the Golgi region (Table I). This finding is rather unexpected since it is suggested that AlF₄⁻ treatment enhances the membrane association of β-COP recruited from a cytosolic pool (Orci et al., 1989).

Occasionally we observed that after incubation with AlF₄⁻, the distribution pattern of β-COP, was similar to that after energy depletion, i.e., β-COP positive aggregates were observed. This observation may indicate that the drug affected the cell energy level before it could activate G-proteins.

Effects of BfA

Dissociation of β-COP from Golgi membranes is one of the earliest effects of BfA treatment of cells (Donaldson et al., 1990). In exocrine pancreatic cells β-COP indeed disappeared almost completely from the membranes in the Golgi region within 10 min of incubation with 1 μg/ml BfA (Fig. 8). At that time part of the Golgi stacks were still present with a morphology comparable with that after energy depletion (Fig. 5), i.e., orderly stacked flattened cisternae with reduced amounts of secretory proteins (Fig. 8). After longer incubation periods with BfA Golgi cisternae fragmented and no intact Golgi stacks were observed (data not shown). Quantitation of β-COP labeling after 10 min in BfA revealed a significant increase of β-COP in the cytoplasm (Table I), proportional to the loss in the Golgi region. Total β-COP labeling of the cell remained constant, as in the other condi-

tions studied, demonstrating that the immunoreactivity of β-COP in cryosections was identical under all circumstances studied.

Discussion

We describe the distribution of the nonclathrin-coated vesicle-associated coat protein β-COP in cells of the rat exocrine pancreas by immuno-electron microscopy. We observed β-COP on clusters of coated buds and vesicles of 50 nm in diameter that were distinct from the clathrin-coated structures in two respects: (a) Clathrin and COP coats had a different thickness; 18 and 10 nm, respectively; (b) the two populations of vesicles were differently distributed within the cell. Most of the label for β-COP (68%) was on the *cis*-side of the Golgi complex, whereas clathrin was predominantly (78%) on the *trans*-side. Although their separation was not complete we can conclude from our observations that in rat exocrine pancreatic cells the two coat systems studied are distributed complementary over the Golgi complex, i.e., the β-COP is located mainly on the *cis*-side whereas clathrin is predominantly on the *trans*-side. This suggests that the early, RER–Golgi transport step, is mediated by COP-coated vesicles while clathrin and associated proteins are involved in exit of the Golgi complex. Half of the β-COP label was distributed throughout the cytoplasm, outside the Golgi region. The majority of this cytoplasmic β-COP was not clearly associated with membranes, except for a small portion (≤1%) that was associated with small clusters of vesicles located between RER cisternae. The amount of cytoplasmic β-COP remained constant under conditions of energy depletion and AlF₄⁻ treatment, and probably represents cytosolic COP complexes (Duden et al., 1991; Waters et al., 1991). So far we have not been able to determine a "soluble" pool of COP complexes biochemically (Robinson and Kreis, 1992). Therefore we can not exclude that part of the cytoplasmic β-COP is associated with RER.

In normal exocrine pancreatic cells, β-COP was present at the *cis*-side of the Golgi complex, on vesicles and buds of the PE. Our observations are compatible with the idea that β-COP is primarily involved in anterograde vesicular transport from RER to Golgi. A hypothetical model for β-COP recycling is outlined in Fig. 9. Buds and tubular extensions are formed at the concave TE membranes. The yeast Sec 23 homologue, described by Orci et al. (1991) to be localized in TA, occurred close to the TE membranes, which is in accordance with the idea that Sec23p, along with other Sec proteins plays a role in budding from the RER of vesicles en route to the Golgi complex (Kaiser and Schekman, 1990). It has been suggested that these Sec proteins are involved in the formation of a scaffold that creates a place for TE membranes to form buds (Orci et al., 1991). The tip of these buds or tubules may carry the binding site for COP coats. Upon coat binding to these sites, vesicles pinch off and migrate to the nearby Golgi stack where they fuse with the *cis*-Golgi. Then the COP coat comes off and recycles to the TA for another round of vesicular transport. Under conditions of energy depletion the budding at the TE membranes is inhibited which is apparent from the decrease of PE in the TA. This is in agreement with early observations that under this condition protein transport is blocked at this location (Jamieson and Palade, 1968; Howell and Whitfield, 1973). The
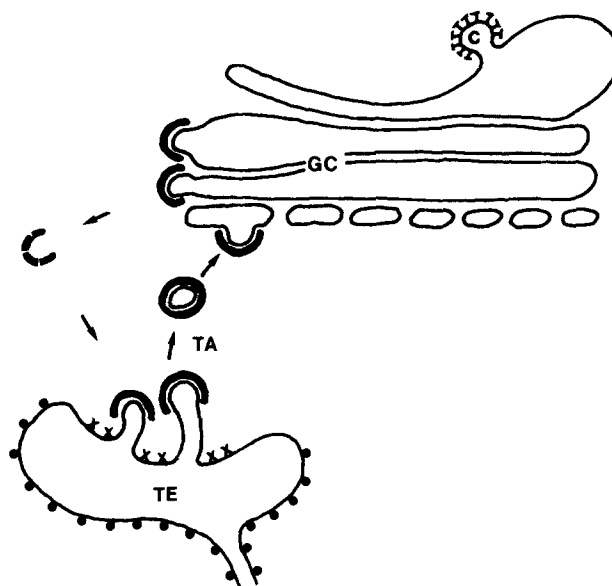
Figure 9. Model for recycling of β-COP in the Golgi area of the exocrine pancreatic cell. As discussed in the text, our observations are in favor of involvement of COP coats (*bold lines*) in anterograde transport between RER and the Golgi complex. Sec23 proteins (x) are present near the membrane at the concave side of the transitional elements (*TE*) of the RER. Here buds or elongated protuberances are formed, from which COP-coated vesicles pinch off and fuse with the Golgi cisternae (*GC*) at the *cis*-side or at the lateral rims. Complete COP coats may recycle to the TA. *c*, clathrin-coated bud.

COP proteins, recycling from the Golgi stack find no new binding sites and accumulate in the aggregates in vicinity of the TA.

The β-COP positive aggregates are probably the same as described by Merisko et al. (1986a) after ATP depletion in guinea pig and rat pancreas. They showed that these aggregates often contained vesicle-free small, globular, cages of coat material. In a subsequent paper Merisko et al. (1986b) also described small amounts of focally distributed clathrin inside these aggregates. We show here that similar structures in rat pancreatic cells do not contain clathrin, but high concentrations of β-COP. Our observations could not confirm the presence of complete coats in the aggregates (Merisko et al., 1986a), probably due to the fuzzy appearance of COP coats in cryosections, which does not allow to recognize them when not attached to membranes. The accumulation of complete COP-coats in the aggregates suggests that they detach and recycle from Golgi membranes more or less intact. This is consistent with a low cytosolic soluble pool of β-COP usually found (Robinson and Kreis, 1992).

As an alternative to a simple RER-Golgi recycling of β-COP as we suggest (Fig. 9), the coat may be involved in a repeated fission–fusion of vesicles at the lateral rims of the cisternae, thus providing a mechanism for inter-cisternal transport. In spite of the predominant presence of β-COP at the *cis*-side our data cannot exclude the possibility that the 17% found at the lateral rims of the Golgi stack is involved in saltatory movement of vesicles along the stack. In fact most reports emphasize β-COP's functioning in such an intra-stack transport rather than its role in the RER-Golgi

step (Orci et al., 1986; Malhorta et al., 1989; Burgoyne, 1992). Another observation with possible relevance in this respect is that the Golgi cisternae fragment and disappear in the presence of BfA and AlF$_4^-$ when COP-mediated influx in the Golgi stack is blocked. This fragmentation of Golgi cisternae must be an active process since its resistance against energy depletion suggests that the stack is a stable structure. A possible explanation for the disappearance of the cisternae in the presence of BfA and AlF$_4^-$ is that subsequent transport events, including the inter-cisternal steps, proceed to some extent, which is only possible if these were β-COP independent. In that case the cisternal pool of material becomes soon exhausted since no new material from the RER arrives. This could explain why only vesicular remnants remain which are either COP coated (in the case of AlF$_4^-$) or not (under BfA conditions). However, other explanations are possible for the disintegration of the Golgi stack. For instance those that account for COP-independent retrograde transport to the RER which is described to proceed under BfA conditions (Lippincott-Schwartz et al., 1990; Klausner et al., 1992). For the time being the mechanism of inter-cisternal Golgi transport remains mysterious and we do not know yet to what extent COP proteins are involved in this process.

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